linked to the cell surface via GPI anchors and serve as co-receptors for ligands of several signalling pathways during development. Notum, a secreted alpha/beta hydrolase, was found to antagonize the signalling of the prototypical *Drosophila* Wnt, Wingless, by shedding glypicans from the cell surface. Biochemical work demonstrated that a mammalian Notum homologue could induce the release of glypicans by GPI cleavage. In zebrafish, we found three notum genes. One homologue, nom1a, is most closely related to mammalian Notum with respect to protein identity and genomic synteny. The expression of nom1a is dynamic and regulated by Wnt/Beta-Catenin signalling. Overexpression of nom1a at multiple stages of development causes changes in gene expression consistent with Wnt/Beta-Catenin inhibition. Additionally, nom1a expression rescues phenotypes induced by Wnt1 and Wnt8 overexpression, while loss of Nom1a enhances these phenotypes. Using loss and gain of function studies, we have shown that Nom1a is required for the proper patterning of the dorsal neural tube. We have found no evidence that Nom1a inhibits the function of Glypican 4 in Wnt/PCP signalling. In contrast, Glypican 3 is a likely target of Nom1a, as alterations of Gpc3 levels alter the severity of Nom1a-overexpression phenotypes. Our analyses suggest that Nom1a has a surprisingly limited set of targets, and we have identified a novel mechanism restricting Wnt/Beta-Catenin signalling.

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Program/Abstract #74
Reduction of cellular sulfation during mouse brain development results in microcephaly marked by neuronal cell death and abnormal neuronal progenitor proliferation
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Sulfation of macromolecules modulates a number of cellular processes including growth factor signaling. The universal sulfate donor in the cell, PAPS, is synthesized by two isoenzymes, PAPSS1 and PAPSS2. PAPSS1 is the predominant isoenzyme expressed in the developing brain. To determine the importance of sulfation during normal brain development, we designed a Cre-mediated, tissue specific RNAi mouse model and generated PAPSS1 brain specific knockdown mice. These mice demonstrate a reduction in PAPPS1 protein and reduced enzymatic activity over control littermates indicating successful in vivo knockdown of PAPSS1. Furthermore, immunostaining for heparan and chondroitin sulfate reveals decreased proteoglycan sulfation. PAPSS1 knockdown mice have microcephaly, with reduced brain size apparent by E14.5 and persisting into adulthood (21.7% volume reduction by MRI). TUNEL and cleaved caspase-3 staining demonstrate that the reduction in cortical size is due to a window of cell death between E10.5 and E12.5. During this period, there is a decrease in β-catenin and an increase in oxidative stress markers. Western blots of E12.5 cortical brain lysates show reduced activated β-catenin suggesting a decrease in Wnt signaling. Interestingly, the window of cell death is followed by a period of proliferation as determined by a greater percentage of BrdU+ and Ki67+ cells. Consistent with this, there is an increase in the level of phosphorylated ERK, an indicator of FGF signaling, in E14.5 and E15.5 brain lysates. These changes in cell signaling pathways in vivo are correlated with changes observed in neuronal stem cell cultures. In sum, this study illustrates the importance of sulfation in modulating signaling pathways during brain development.

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Program/Abstract #75
The role of glycosaminoglycans in FGF diffusion during lacrimal gland branching morphogenesis
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Glycosaminoglycans (GAGs) play a central role in embryonic development by regulating the movement and signaling of morphogens. We have previously demonstrated that GAGs are the co-receptors for Fgf10 signaling in lacrimal gland epithelium, but their function in Fgf10-producing periocular mesenchyme is still poorly understood. In this study, we have generated a mesenchymal ablation of UDP-glucose dehydrogenase (Ugdh), an essential biosynthetic enzyme for GAGs. Although Fgf10 is expressed normally in the Ugdh mutant mesenchyme, it fails to elicit FGF signaling response or budding morphogenesis in the perspective lacrimal gland epithelium. This is confirmed in explant culture, where the mesenchymal Ugdh deletion also prevents FGF10-absorbed beads from inducing lacrimal gland budding. In contrast, the Ugdh mutant phenotype can be rescued by constitutive Ras activation in the lacrimal gland epithelium but not in the mesenchyme. Previous studies have indicated that reducing GAGs-FGF binding leads to increased FGF diffusion and ectopic FGF signaling activation, while our data show that eliminating GAGs–FGF interaction in mesenchyme abolishes FGF signaling. Taken together, these results demonstrate a biphasic control of FGF signaling by GAGs in regulating FGF gradient.

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Program/Abstract #76
Sulfatases modulate FGF and hedgehog signaling during zebrafish organogenesis
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Heparan sulfate proteoglycans (HSPGs) are heavily glycosylated extracellular or membrane-associated proteins, with sulfated domains that can bind to many growth factors, modifying their activity or diffusion. The pattern of HSPG sulfation can be modified by extracellular sulfatases, which remove specific sulfates from the heparan sulfate chains. We have examined the role of two 6-O-endosulfatases (sulf1 and sulf2) during organogenesis of zebrafish embryos. We generated anti-sense morpholino oligonucleotides to the translation start site and to a splice junction for both sulf1 and sulf2, and injected these into embryos at the 1–4 cell stage. In sulf1 morphants, fish had moderate trunk muscle defects that produced U-shaped somites and abnormal muscle fibers, hydrocephalus over the hindbrain, microophthalmia, lack of pectoral fin development, malformation of the ear, and improper migration of the lateral line. These phenotypes correlate well with expression of sulf1 mRNA in somites, ear and developing neural tube. sulf2 morphants showed midbrain hydrocephalus, but had normal ear and somite development. Fish injected with both sulf1 and sulf2 morpholinos had extensive hydrocephalus over both the midbrain and hindbrain, as well as the effects seen in the individual morphants. In situ hybridization of downstream target probes for Wnt, FGF and hedgehog signaling showed that knocking down sulfatase expression modulated FGF and hedgehog signaling in the developing nervous system and somites, while Wnt signaling appeared to be normal. These results suggest that sulfatases play an essential role in precisely regulating the

active domains of FGF and hedgehog signaling during zebrafish organogenesis.

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Program/Abstract #77
Shh is required for the maintenance of postnatal mouse intervertebral disc

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Introduction: Degenerative disc disease is a major cause of lower back pain. However the molecular signals that control normal growth and differentiation of the disc are not well defined. We hypothesize that nucleus pulposus (NP) cells, which originate from the embryonic notochord, provide signals that control growth and differentiation of the postnatal disc. Methods: Postnatal day 4 mouse IVDs were cultured in serum-free DMEM Ham/F12 medium at 37 °C in 5% CO_2 for 2–5 days on type IV collagen-coated cell culture inserts. Cyclopamine (250 μM) was used as a Shh antagonist, and its specificity was tested by add-back experiments using 100 nM recombinant Shh (rShh). Cell proliferation was assayed using pulse-labelling with BrdU. 6 μm thick cryosections were immunostained (IHC) using antibodies specific to IVD differentiation markers, and Cy5 conjugated secondary antibodies. Imaging was carried out using confocal microscope. To test the role of Shh in vivo, triple transgenic mice [(tetO)7CMV-cre:R26 rtTA:Shhflx/flx] were injected with 2 μg of doxycycline on postnatal day 4 to conditionally delete Shh, and the lumbar spine was collected 5 days later. Results were compared to vehicle treated controls. Results: Both cyclopamine treatment in vitro and conditional Shh knockout in vivo caused rounding up and aggregation of the NP cells, and loss of orientation of the layers of the annulus fibrosus (AF). There was also a loss of expression of both NP and AF differentiation markers. BrdU incorporation studies showed loss of proliferation of NP cells. IHC for activated caspase-3 showed increased levels of apoptosis of NP cells rShh replacement and reversed the cyclopamine phenotype in vitro. Conclusions: These results show that Shh is essential for postnatal growth and differentiation of both the NP and AF. Significance: Identification of signals controlling IVD growth and differentiation offers the opportunity for the development of biological repair as an alternative to surgery.

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Program/Abstract #82
XTRIC-8, a protein required for proper neural crest formation

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The neural crest is a transient embryonic cell population that migrates extensively to various parts of the embryo, where it differentiates into diverse derivatives, including most of the cranio-facial skeleton and peripheral nervous system. Ric-8 has been characterized as a guanine nucleotide exchange factor (GEF) for heterotrimeric G proteins, and thus an activator for those proteins signaling pathways. In this work, we determined that in Xenopus tropicalis embryos, XtRic-8 is expressed in neural crest cells before and after the migration step takes place and later in neural crest derivatives as craniofacial arcs and otic vesicle. In order to study the function of XtRic-8 in neural crest formation, loss-of-function experiments in two-cell stage with antisense morpholino were carried out, resulting in an altered expression of neural crest markers. This result suggests that XtRic-8 is necessary for proper neural crest specification and migration. Loss-of-function experiment effects in neural crest formation could be due to the fact that XtRic-8 is also probably required for normal development during previous stages. On the other hand, transplantation assays, resulting in migration defects and cartilage staining assays, resulting in craniofacial defects, confirmed that XtRic-8 is required for proper neural crest formation.

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Program/Abstract #83
Tetraspanin18 restricts neural crest migration by modulating Cadherin6B mRNA and protein levels

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Unlike typical neuroepithelial cells in the developing central nervous system, neural crest cells undergo an epithelial to mesenchymal transition (EMT), detach from the neural tube, and migrate great distances to give rise to diverse structures, such as the peripheral nervous system, melanocytes and craniofacial skeleton.